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IBIC, KASETSART UNIVERSITY, P.O. BOX 1084
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EMERGENCY INDUCTION OF PARTURITION IN BUFFALOES

S.P. Shukla, Anand Pandey and S.P. Nema

ABSTRACT

In the present study, 17 buffaloes were treated for emergency induction of parturition with Dexamethasone alone or in combination with PGF_2 alpha. It was concluded that Dexamethasone alone or in combination with PGF_2 Alpha can be used successfully for emergency induction of parturition in buffaloes although there was a higher incidence of dystocia, retention of foetal membranes and mortality of calves, which needs to be minimized by careful follow up, critical observation and prompt assistance. Further therapeutic trials are needed to minimize the incidence of retention of foetal membranes and dystocia.

Keywords: parturition, dexamethasone, foetal membranes

INTRODUCTION

It is believed that initiating signals for hormonal changes that terminate pregnancy come from the foetus through an increased secretion of cortisol. The first report on the use of corticoids to induce premature parturition in cattle was reported by Adams (1969). The ability of synthetic corticosteroids to induce parturition in cattle has been found applicable to synchronize calving for better and convenient calving management. This not only minimizes the dystocia problem but also facilitates therapeutic termination of pregnancy for various clinical reasons. In the present investigation parturition, was induced in buffaloes using corticosteroid alone or in combination with PGF_2 alpha for therapeutic termination of pregnancy for clinical reasons.

MATERIALS AND METHODS

In the present study, emergency parturition was induced in 17 buffaloes in advanced pregnancy belonging to local farmers to avoid total loss. The therapeutic termination of pregnancy was done 5-20 days before completion of the full term of gestation. The animals were randomly divided into two groups.

Group I consisted of six buffaloes having gestation length less than 300 days. They were treated with Dexamethasone 20 mg + PGF_2 Alpha 25 mg intramuscular only once.

Group II consisted of 11 buffaloes having gestation length more than 300 days. They were treated with dexamethasone 20 mg intramuscular once only.

The animals were observed for the duration of parturition (hours), occurrence of dystocia (%), incidence of retention of foetal membranes (%), live calves born (%), and sex ratio of calves born. The data was analysed as per the method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The observations in relation to induction of parturition in buffaloes (mean $\pm$ SE) is reported in the table. In the present study, emergency parturition was induced in 17 buffaloes. It was recorded that all the buffaloes (100%) calved in between 18.5 h and 67.25 h. Phogat *et al*. (1994) also reported that

Department of Animal Reproduction, Gynaecology & Obstetrics,
College of Veterinary Science & Animal Husbandry, MHOW 453 446, (M.P.) India.

Dexamethasone successfully induced parturition in buffaloes.

In the present study, it was recorded that the overall duration between commencement of treatment to induction of parturition was 26.58±2.57 h. The present findings are in close accordance with the findings of Nakao *et.al* (1996) who reported the average interval of 31.5 hours after the treatment given for induction of parturition in cattle. The duration was significantly greater (P<0.01) in Group I (Dexamethasone + PGF_2 alpha) in comparison to Group II (Dexamethasone). The reason for greater duration required in Group I may be because of lower gestation length (less than 300 days) in the buffaloes of Group I.

In the present study, overall incidence of dystocia after the treatment for induction of parturition was recorded to be 17.64%. The overall occurrence of dystocia was significantly higher (P<0.01) in Group I in comparison to Group II. It was recorded that the occurrence of dystocia was more common in the cases in which the foetus was born dead.

In the present investigation, the occurrence of dystocia was much higher (17.64%) than the normal range (3.3 percent as reported by Williams, 1943). The reason may be incomplete preparation of birth canal for parturition due to shorter gestation length in the buffaloes treated for induction of parturition). Some investigators have reported that the cows that were induced for parturition more than two weeks pre-maturely suffered a higher incidence of dystocia than normal cows; this may be due to incomplete uterine maturation coupled with malpresentation of foetus.

It was recorded that the overall incidence of retention of foetal membranes was higher (64.70%) than the normal limits. Peters and Poole (1992) and Kask *et al.* (2000) have also recorded higher incidences of retention of foetal membranes (RFM) in cows after induction of parturition with prostaglandin F_2 alpha.

Retention of foetal membranes was significantly higher in Group I in comparison with the Group II. The higher incidence of retained foetal membranes in Group I may be because of shorter gestation length resulting in improper maturation of placenta. The calf mortality in the present study was 11.77 percent, which was higher than the permissible limit. It was observed that more live foetuses were found in treatment Group II in comparison to Group I. The high incidence of calf mortality appears to be due to premature placental separation and increased frequency of uterine inertia. However, calf mortality may be reduced to some extent by careful observation and prompt assistance.

It was concluded that Dexamethasone alone or in combination with PGF_2 alpha can be used for emergency induction of parturition. With the latter treatment there were higher incidences of dystocia, retention of foetal membranes and mortality of calves which needs to be minimized by conducting various trials.

Table. Observations in relation to induction of parturition in buffaloes (Mean ± SE).

Observations	Groups		
	Dexamethasone + PGF_2 alpha (Group I, N-6)	**Dexamethasone (Group II ,n=11)**	**Both groups (n=17)**
Duration of parturition (h)	37.83 ± 2.61**	20.45± 2.06	26.58±2.57
Occurrence of dystocia (%)	50.00**	-	17.64
Occurrence of retention of placenta	100**	45.45	64.70
Live calves (%)	66.66	100	88.23**
Sex ratio	3:3	3:8	6:1
F : M	1:1	1:2.66	1:1.83

** Significant (P<0.01)

*Continued on page 160

BIOMETRY OF OVARIES AND FOLLICULAR COUNT IN CYCLING AND NON-CYCLING NAGPURI BUFFALOES (*BUBALUS BUBALIS*)

W.A.A. Razzaque, S.K. Sahatpure, C.H. Pawshe and S.V. Kuralkar

ABSTRACT

Biometry and follicular population on ovaries of Nagpuri buffaloes procured from the Civil Slaughterhouse, Akola were studied. The dimensions were recorded using a vernier caliper. The visible follicles were grouped/categorized into three groups, viz., Group I small-size (1-3 mm), Group II medium size (3-5 mm) and Group III large size follicles ($\geq$ 5 mm) in diameter. In the present study, the average weights of ovaries in cycling and non cycling buffaloes differed significantly (P<0.01%). In the present study, the average number of small- medium- and large-size follicles recorded were 3.30 $\pm$ 0.30, 1.77 $\pm$ 0.25 , and 1.22 $\pm$ 0.22, respectively, in cycling buffaloes as compared to3.43 $\pm$ 0.28, 1.54 $\pm$ 0.18, and 0.89 $\pm$ 0.11 follicles respectively in non-cycling buffaloes.

Keywords: biometry, buffalo ovaries, follicular population

INTRODUCTION

The world buffalo population is estimated to be approximately 166.4 million, and out of this, 161.4 million (96.99%) are found in Asia. India alone has 94.13 million buffaloes, which is 56.60% of the world buffalo population. Most of the Indian buffalo population is of nondescript type and are unique in there adaptation to the agro-climatic conditions of their habitats and management practices. This animal represents a unique combination of genes acquired over the years by natural selection and represents a major component of the world diversity.

Buffaloes play a prominent role in livestock production and the economy in India and contribute more than 50% of total milk production (Anon 2003). However, their productivity is limited by poor reproductive efficiency.

It is essential to establish the norms of reproductive parameters. One such parameters in the buffalo is the reproductive potential expressed as the number and quality of follicles. It is important to evaluate this parameter as a co-relation between number of primordial follicles and Graafian follicles $\geq$1 mm (Sattergren, 1964). Available reports are suggestive of lower follicular populations in buffalo ovaries. However, knowledge of the structure of the follicle and folliculogenesis to optimize the reproductive efficiency in buffaloes is scanty though efforts have been made by Bhalla *et al*. (1964) and Sane *et al.* (1965). The objective of the present study was to estimate the reproductive potential of the buffalo by quantitative investigation of the follicular system of the ovaries in cycling and non-cycling Nagpuri buffaloes as it forms the basis for investigations on the fertility and improving productivity of the native buffalo breed. As yet, no study has been reported on the relation between follicular number and fertility in Nagpuri buffaloes, and very little information of this breed is available in the literature.

MATERIALS AND METHODS

The present study was conducted on 96 ovaries (48 cycling and 48 non-cycling) of Nagpuri buffaloes collected from a local municipal abattoir and transported to the laboratory in physiological

Department of Animal Reproduction and Gynaecology, Post Graduate Institute of Veterinary and Animal Sciences MAFSU, Krishinagar, Akola, Maharashtra-444 104, India.

saline at 30-38°C within 30 minutes of slaughter. Based on the characteristics of the corpus luteum, the oestrus cycle was fixed into two cycles. Length, width and height of the ovaries were measured as pole to pole, surface to surface and hilus to the free border, respectively, with the help of a vernier caliper. Linear measurements of the ovaries were made with a vernier caliper using the average of three measurements as per Bhattacharya and Luktuke (1960).

Length: The length of the ovary was taken as the distance from the anterior pole to the posterior pole along an axis parallel to the ovarian mesenterial attachment (base).

Width: Width of the ovary was taken as the greater distance from the medial to the lateral surfaces or borders.

Thickness: Thickness of the ovary was recorded as the greatest distance along an axis vertical to the longitudinal axis (base) at its center or distance from attached to the free borders expressed in cm.

Weight: Weight of the ovaries was taken on the electronic (digital) mono pan balance and expressed in g.

Graafian follicles in each ovary were counted as criterion of follicular activity and were measured *in situ*; only those clearly visible on the ovarian surface were studied. The diameters of the follicles and corpus luteum were measured with a vernier caliper as per Bhattacharya and Luktuke (1960).

The visible follicles were identified and counted in each ovary, and their diameters were measured with a vernier caliper and classified according to their size (diameter) adopting the standards of small: 1-3 mm; medium: 3-5 mm; and large: ≥5 mm diameter. The data collected were analyzed by ANOVA and standard procedures described by Snedecor and Cochran (1967). Values were presented as mean ± SE.

RESULTS AND DISCUSSION

The average weights of ovaries in cycling and non-cycling buffaloes were 2.71 ± 0.13 g and 3.36 ± 0.19 g, respectively. The present findings are comparable to earlier findings of Kaikani (1974); Danell (1987); Naiar and Sarma (1999) recorded 2.35 ± 0.06 g and 2.27 ± 0.04 g; 2.52 ± 1.18 g and 2.48 ± 0.90 g in non-cyclic and 3.94 ± 1.29 g and 3.59 ± 1.54 g in cyclic surti buffaloes.

The average measurements of ovaries of Nagpuri buffaloes are presented in Table 1. The average length, width and thickness of ovaries were found to be 2.21 ± 0.13 cm, 1.39 ± 0.03 cm and 1.49 ± 0.04 cm, respectively, in cycling buffaloes as compared to 2.45 ± 0.07 cm, 1.52 ± 0.04 cm and 1.68 ± 0.05 cm, respectively, in non-cycling buffaloes. These findings are comparable to the earlier reports of Parkale and Hukeri (1989), Danell(1987), Jainudeen (1983), Chauhan and Adamu (1990) in cows, Amle *et al*. (1992), Ezakial and Quyaam (1996), Kumar *et al*. (2003), Bhalla *et al*. (1964) and Sane *et al*. (1965) in buffaloes.

In the present study, the average number of small-size surface follicles counted was 3.30 ± 0.30 in cycling buffaloes as compared to 3.43 ± 0.28 follicles in non-cycling buffaloes. The difference was not significant. The present findings are comparable to those of Danell (1987), who reported 3.6, 4.8 and 5.0, 2.4 small-size surface follicles in cycling and non-cycling Surti buffaloes. The surface count of small-size follicles was less than the counts reported by Sattergren (1964), Fitzpatrck and Entwistle (1997) and Pierson and Gunther (1987). This low number of small-size follicles recorded in the present study may be due to plane of nutrition, to differences in methodology of counting and possibly to species differences. In Table 2, it can be seen that the average number of medium-size surface follicles was greater (1.77 ± 0.25) in cycling buffaloes as compared to 1.54 ± 0.18 follicles in non-cycling buffaloes, the difference being non-significant. These findings are in agreement with Danell (1987); Mittal and Madan (1992) who reported on an average of 1.1 and 2.2 follicles of 3-3.9 mm size in cycling and non cycling buffaloes, respectively. The number of follicles observed in the present study was comparatively lower and hence not in agreement with the earlier observations of Pierson and Ginther (1987). Fitzapatrick and Wistle (1997) in Holstein heifers and cows reported an average 5.4 ± 0.4 and 16.00 ± 0.2 follicles, respectively. The present findings are in closer to the earlier observations of

Pattabiraman and Kathireson (1993), who reported on an average of 2.20 and 3.60 in estrus and anestrous ovaries and Ali *et al.* (2003) who reported 1.28 ± 1.5, 2.26 ± 2.8, 1.71 ± 1.8 and 2.35 ± 1.8 follicles in oestrus, late dioestrus, early dioestrus and metestrous ovaries, respectively. The pooled average large-size surface follicles recorded in the present study was comparatively higher (1.22 ± 0.22) in cycling buffaloes than the 0.89 ± 0.11 follicles in non-cycling buffaloes. The present findings for average large-size follicles in cycling buffaloes are similar to the earlier findings of Ali *et al.* (2003), who reported on an average of 1.28 ± 1.5 follicles in proestrus buffaloes. These findings are similar and hence in agreement with earlier findings 1.6 ± 0.2 Pierson and Ginther (1987).

Table 1. Average weight and dimensions of ovaries.

	No of observations	Average weight (g)	Length (cm)	Width (cm)	Thickness (cm)
Cyclic buffaloes	N =48	2.71^{b}± 0.13 (0.87-4.87)	2.21^{b}± 0.06 (1.10-2.90)	1.39± 0.03 (0.80-1.80)	1.49± 0.04 (0.90-2.20)
Non-cyclic buffaloes	N =48	3.36^{a}± 0.19 (1.20-7.49)	2.45^{a}± 0.07 (1.20-3.40)	1.52± 0.04 (1.00-2.20)	1.68± 0.05 (1.00-2.40)

Means bearing different superscripts in a weight column differ significantly (P<0.01)
Means bearing different superscripts in a weight column differ significantly (P<0.05)
Figures in parenthesis indicates range

Table 2. Average number of follicular population on ovarian surface.

	No of observations	Average No of small size surface follicles	Average No of medium size surface follicles	Average No of large size surface follicles
Cyclic buffaloes	N =48	3.33± 0.30 (0- 6)	1.77± 0.25 (0- 6)	1.22± 0.22 (0- 4)
Non-cyclic buffaloes	N =48	3.43± 0.28 (0- 7)	1.54± 0.18 (0- 4)	0.89± 0.11 (0- 3)

Figures in parenthesis indicates range

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MULTI-TRAIT SELECTION FOR GENETIC IMPROVEMENT IN INDIAN BUFFALOES

Sunil Kumar, M. C. Yadav and R.B. Prasad

ABSTRACT

Records on 1753 buffaloes belonging to three genetic groups i.e. Murrah, graded Murrah and Nili Ravi from six military dairy farms of north India were used for the study. Variables considered for construction of selection index were AFC (age at first calving in months), WFC (weight at first calving in kg), FLP (first lactation period in days), FCI (first calving interval in days), FLMY (first lactation milk yield in kg), AFLMY (average milk yield per day of first calving interval in kg), PFL (profit in first lactation in Rs.) and APFL (average profit per day of first calving interval in Rs.). In all, 23 standard multi-trait selection indices were constructed using different combinations of the above mentioned traits. Maximum genetic improvement in FLMY (433.67 kg) was by the index incorporating all the eight traits while PFL was maximum (Rs.34381.0) by the index 7 which incorporated only three traits i.e. AFLMY, PFL and APFL. Based on expected genetic economic gain it was concluded that the selection index for buffaloes could be either 7607.6 AFLMY +0.063 PFL + 52715.5 APFL or -117.64 AFC+ 3.21 WFC + 26.60 FLP + 118.50 FCI – 4.88 FLMY – 26180.0 AFLMY + 0.066 PFL = 31782.0 APFL.

Keywords: profit, selection, index, economic gain, intensity

INTRODUCTION

Since the unit of selection is always an individual and not a trait, the animal breeder has to improve several economically important characters of animals simultaneously to increase profitability. The economic conditions of dairying demand that not only the selected animals should be high yielders but should also be profitable. Although the efficiency with which an animal converts raw inputs into finished product is an important characteristic in determining the economy of the animals, there may be other variables also which readily spell out the overall economic efficiency. The economics of buffalo breeding depends not only on production performance but also on some other important traits of growth and reproduction.

The characters to be included in multi-trait selection is a matter of discussion but the trait of first lactation is considered better to reduce the generation interval as against the traits of second or third lactations because by the time information is collected, the animal has become too old to produce further. Various workers (Sharma and Basu, 1986 and Gupta *et al*., 1991) have used body weight of buffalo at 6 months,1 year, age at first calving, weight at first calving, first lactation milk yield, first calving interval etc but not incorporation of first lactation profit and average profit per day of first calving interval.

Similarly various workers have estimated relative economic value of the traits to be incorporated in selection index to get desired gain but Panse *et al*. (1967) and Gurnani (1968) have indicated that relative economic value for the traits included in the index does not affect much the efficiency of selection index. Even 100 % error in economic value of the trait would reduce the efficiency of the index by only 4% (Panse *et al*., 1967). Gupta *et al*. (1991) also recommended that

Department of Animal Sciences, Hill Campus, G.B. Pant University of Agriculture and Technology, P.O. Ranichauri - 249 199, Distt. Tehri Garhwal, Uttaranchal, India.

to get desired gain in important economic traits, their relative economic values are not required.

Therefore, an effort has been made to understand the importance of production, reproduction and profit traits of first lactation in selecting buffaloes on organized farms of north India.

MATERIALS AND METHODS

1 Data: Records on 1753 buffaloes belonging to three genetic group i.e. Murrah, Graded Murrah and Nili Ravi from six military dairy farms located between latitude of 23° 10' to 30° 56' N and longitude of 75° 52' to 81° 33' E in India were used for the study. All the animals were maintained under standard management conditions. Variables considered for each buffalo were first lactation traits like AFC (age at first calving in months), WFC (weight at first calving in kg), FLP (first lactation period in days), FCI (first calving interval in days), FLMY (first lactation milk yield in kg), AFLMY (average milk yield per day of first calving interval in kg), PFL (profit in first lactation in Rs) and APFL (average profit per day of first calving interval in Rs).

In order to obtain adequate data sets for statistical analysis, records used in the study were edited as follows: buffalo that produces milk for more than 150 days and that, dried under normal physiological condition were included. Abortions and other pathological causes which affected the first lactation data were not included in the study.

The profit characters (PFL and APFL) were calculated as per Sunil Kumar *et al.* (2006) and are summarized in Table1.

Individual feeding cost was obtained from average intake of 0.025 kg of dry matter per kg body weight of buffaloes per day and the amount of concentrate requirement was taken as 0.40 kg/kg of milk produced as feeding and management was in groups.

2 Selection Index: The index or breeding value was $I = b_1x_1 + b_2x_2 + \ldots + b_n x_n$ was constructed by solving normal simultaneous equations for each trait as per Henderson (1963) as under:

$$[b] = [P]^{-1} [G] [a]$$

where [b] is a column vector of partial regression coefficients of X's in the trait, [P] is a variance-covariance matrix of phenotypic values, [G] is a variance-covariance matrix of genetic values and [a] is a column vector of economic weights of 1.00 for each trait.

In order to find the most suitable and efficient selection index following criteria were used:

The change in aggregate genetic economic gain:

$$\triangle H = \Sigma A \triangle P_i$$

Accuracy of selection

$$r_{TI} = \sigma T I/(\sigma T \sigma I)$$

Standard deviation of index:

$$\sigma T = \surd (b' P b)$$

Heritability estimate of index:

$$h^2_I = s^2 g/ s^2 P = [(b' G b)/ (b' P b)]$$

Table1. Average economic data for six military dairy farms in northern India.

Concept	Price
Base milk price (Rs./kg)	10.00
Cost of concentrate (Rs./quintal)	350.00
Cost of dry matter fed (2/3 green + 1/3 dry fodder fed to the buffalo) (Rs./quintal)	177.78
Interest on cost of rearing of buffalo (% of rearing cost)	15.00
Cost of insemination (Rs./insemination)	20.00
Calf price at day old age (Rs./ calf)	200.00
Labour cost (one labour per 14 buffalo per day) (Rs./buffalo/year)	1303.05
Other costs per buffalo (Rs. /buffalo/year)	382.14
Cost of rearing one heifer to 3 years of age (Rs./heifer)	19777.77

where $\triangle P_i$ = expected genetic gain in i^{th} trait due to unit change in index in standard deviation form

$= (\Sigma b_i G_{ij} / \sigma I) i$,

A = relative economic value of the i^{th} trait included in the index, which was taken as 1.00,

b_i=column vector of coefficient of index,

G_{ij} =genetic value of the i^{th} trait,

σ T=standard deviation of the index = ($\checkmark b' P b$)

i = intensity of selection (0.497 if 70% of buffaloes to be retained for further breeding in each generation,

σ T I = Covariance between index and aggregate genotypic value,

σ I=Standard deviation of aggregate genotypic value= $\checkmark (b' G b)$

RESULTS AND DISCUSSION

Least square means of the traits used for selection index in the herd is given in Table 2. A total of 23 standard selection indices were constructed using different combinations of first lactation traits. Selection indices along with index coefficients (b_i) and expected genetic gain in individual trait in buffaloes are presented in Tables 3 and 4, respectively. The maximum genetic improvement in FLMY (433.67 kg) would be expected by the index I_{23} which incorporated all the eight traits followed by I_{15} (10.53 kg) which incorporated only WFC, FLP, FLMY and AFLMY traits (Table 4).

The genetic improvement in PFL was maximum (Rs.17944.40 in index $I_{7,}$ which incorporated AFLMY, PFL and APFL, followed by I_{23}, where all the traits were considered for selection index. The result of expected genetic gain in FCI was maximum (reduction of 74.3 days) if I_{23} was applied. I_{23} would be able to decrease AFC, WFC, FLP, FCI but will increase first lactation milk yield, average milk production per day of first calving interval, profit in first lactation and average profit per day of first calving interval (Table 4)

Accuracy of selection: The results of accuracy of selection (Table 5) indicated that maximum accuracy (0.346) could be obtained if all the eight traits are incorporated in the selection index followed by I_{21} and I_{22} where these two indices were 82% efficient as compared to I_{23}. The accuracy of selection for indices incorporating various combination of traits were lower than that reported by Bhalaru and Dillon (1981), Singh and Prakash (1986), Sharma and Singh (1990), Dutt (1991) and Kuralkar (1996). Traits like profit in first lactation and average profit per day of first calving interval have not been inserted in developing selection indices by other workers. Therefore, no comparison is possible for the accuracy measured by selection indices incorporating these two traits except Sharma and Basu (1986) who recommended selection based on first lactation milk yield and first lactation profit although the accuracy was lower. The accuracy was very poor for the index incorporating only two traits

Table 2. Least squares means and standard errors of the traits under study.

S. no.	Traits	Mean	Standard error
1	Age at first calving (months)	41.12	0.35
2	Weight at first calving (kg)	483.588	2.962
3	First lactation period (days)	302.75	2.66
4	First calving interval (day)	471.99	5.74
5	First lactation milk yield (kg)	1818.41	21.26
6	Average milk yield per day of first calving interval (kg)	3.95	0.07
7	Profit in first lactation (Rs.)	1992.02	370.79
8	Average profit per day of first calving interval (Rs.)	5.43	0.08

at a time. Therefore, the best index developed was I = -117.64 AFC+ 3.21 WFC + 26.60 FLP + 118.50 FCI – 4.88 FLMY – 26180.0 AFLMY + 0.066 PFL + 31782.0 APFL.

Standard deviation of index: The results of standard deviation (Table 5) indicated minimum for index I_1 (0.428) and maximum for index I_7 (17940.967). In general, the standard deviations were higher for the index incorporating PFL and APFL as compared to those incorporating AFC, WFC, FLP, FCI, FLMY and AFLMY. This was due to the fact that there was very high gap between losses to profits by different buffaloes.

Table 3. Selection index coefficients in different combinations of first lactation traits.

Combination of traits	Index coefficients							
	AFC (1)	WFC (2)	FLP (3)	FCI (4)	FLMY (5)	AFLMY (6)	PFL (7)	APFL (8)
I_1 (6,8)	-	-	-	-	-	1.632	-	-1.254
I_2 (5,7)	-	-	-	-	-2.477×10^{-2}	-	3.450×10^{-4}	-
I_3 (4,5)	-	-	-	0.017	-4.279×10^{-1}	-	-	-
I_4 (3,5)	-	-	0.107	-	-7.902×10^{-3}	-	-	-
I_5 (1,2)	-0.328	9.384×10^{-2}	-	-	-	-	-	-
I_6 (2,5)	-	7.366×10^{-2}	-	-	-3.115×10^{-3}	-	-	-
I_7 (6,7,8)	-	-	-	-	-	7607.6	0.063	52715.5
I_8 (5,6,7)	-	-	-	-	2.488	-1586.65	0.083	-
I_9 (4,5,7)	-	-	-	-0.437	0.021	-	-3.476×10^{-2}	-
I_{10} (3,4,5)	-	-	0.106	1.086×10^{-3}	4.975×10^{-3}	-	-	-
I_{11} (3,5,6)	-	-	0.155	-	-0.018	4.923	-	-
I_{12} (2,4,5)	-	0.102	-	0.031	7.942×10^{-3}	-	-	-
I_{13} (1,2,3,4)	-1.876	0.061	-0.212	0.166	-	-	-	-
I_{14} (1,2,4,5)	-4.318	0.118	-	0.042	0.018	-	-	-
I_{15} (2,3,5,6)	-	0.083	0.233	-	-0.012	7.344	-	-
I_{16} (2,3,7,8)	-	-1.141	-0.449	-	-	-	-7466.86	0.076
I_{17} (3,5,7,8)	-	-	-2.368	-	0.234	-	-7.593×10^{4}	-58.380
I_{18} (2,3,4,5,7)	-	-1.397	3.926	-2.606	-0.431	-	-8.025×10^{-3}	-
I_{19} (1,2,5,6,7)	633.787	242.809	-	-	228.492	-144741.0	8.061	-
I_{20} (1,2,3,7,8)	105.542	-1.039	8.331	-	-	-	0.018	46.534
I_{21} (1,2,3,4,5,7)	-106.150	-0.146	-0.544	-8.929	-0.992	-	-0.072	-
I_{22} (3,4,5,6,7,8)	-	-	202.620	-356.651	13.830	-1.460×10^{8}	-0.073	999467.0
I_{23} (1,2,3,4,5,6,7,8)	-117.649	3.214	26.609	118.506	-4.889	-26180.0	0.066	31782.0

Heritability of index: Heritability of the index ranged between 0.011 to 0.280 (Table5). Highest heritability of the index was for I_{19}, which was only 25% efficient as compared to I_{23}. Heritability estimates of I_2, I_3, I_4, I_6, I_9 and I_{16} were very low, ranging between 0.011 to 0.023 whereas that of I_8, I_{13}, I_{18} and I_{23} ranged between 0.119 to 0.187 (Table 5).

Expected aggregate economic gain in genotype: The expected aggregate economic genetic gain would be highest (Rs. 8916.66) with use of index I_7, incorporating FLMY, PFL and APFL traits, followed by I_{23} (Rs. 4187.99) and I_8 (Rs. 594.921). All these selection indices incorporated either PFL or APFL or both traits. Sharma and Basu (1986). Singh and Prakash (1986), El-Arian and Tripathi (1990), and Sharma and Singh (1990) reported higher aggregate genetic gain by incorporating AFC, FLMY and FCI than the present report.

Table 4. Expected economic genetic gain in component traits due to different selection indexes.

Combination of traits	Expected genetic gain in each trait							
	AFC (1)	WFC (2)	FLP (3)	FCI (4)	FLMY (5)	AFLMY (6)	PFL (7)	APFL (8)
I_1 (6,8)	-	-	-	-	-	1.564	-	1.993
I_2 (5,7)	-	-	-	-	1.207	-	9.642	-
I_3 (4,5)	-	-	-	-1.298	1.120	-	-	-
I_4 (3,5)	-	-	0.807	-	4.763	-	-	-
I_5 (1,2)	-0.385	5.520	-	-	-	-	-	-
I_6 (2,5)	-	-0.718	-	-	4.103	-	-	-
I_7 (6,7,8)	-	-	-	-	-	-1.616	17944.40	-4.654
I_8 (5,6,7)	-	-	-	-	-0.406	-0.069	1196.89	-
I_9 (4,5,7)	-	-	-	0.881	-2.460	-	45.349	-
I_{10} (3,4,5)	-	-	1.541	3.353	5.086	-	-	-
I_{11} (3,5,6)	-	-	-2.343	-	4.928	-0.691	-	-
I_{12} (2,4,5)	-	2.744	-	4.761	6.386	-	-	-
I_{13} (1,2,3,4)	0.973	8.996	8.684	-2.238	-	-	-	-
I_{14} (1,2,4,5)	-0.232	3.991	-	-12.842	-4.099	-	-	-
I_{15} (2,3,5,6)	-	1.975	-0.688	-	10.532	-3.924	-	-
I_{16} (2,3,7,8)	-	-0.370	-1.194	-	-	-	72.852	-0.483
I_{17} (3,5,7,8)	-	-	2.362	-	-3.706	-	107.564	6.214
I_{18} (2,3,4,5,7)	-	-2.882	-14.509	-1.597	7.863	-	312.522	-
I_{19} (1,2,5,6,7)	1.466	6.165	-	-	3.167	-15.392	10795.1	-
I_{20} (1,2,3,7,8)	2.567	3.202	6.183	-	-	-	-782.997	5.005
I_{21} (1,2,3,4,5,7)	-2.347	-9.383	-11.133	2.992	-14.127	-	1112.420	-
I_{22} (3,4,5,6,7,8)	-	-	-1.497	-7.915	-5.521	-90.404	3438.1	43.841
I_{23} (1,2,3,4,5,6,7,8)	-1.232	-2.223	-3.221	-74.317	433.67	2.977	7979.66	0.109

Table 5. Accuracy of selection (r_{TI}), standard deviation of index (SD), heritability estimate of index (h^2) and expected aggregate genetic economic gain ($\triangle$H) by each index.

Indices	r_{TI}	SD	h^2	ΔH(Rs)	Relative efficiency on r_{TI} basis (%)
I_1 (6,8)	$0.359x10^{-3}$	0.428	0.124	0.212	0.08
I_2 (5,7)	$0.911x10^{-2}$	10.849	0.030	5.392	2.63
I_3 (4,5)	$0.203x10^{-2}$	2.418	0.011	1.201	0.57
I_4 (3,5)	$0.473x10^{-2}$	5.631	0.023	2.798	1.35
I_5 (1,2)	$0.495x10^{-2}$	5.904	0.109	2.934	1.41
I_6 (2,5)	$0.405x10^{-2}$	4.822	0.013	2.396	1.15
I_7 (6,7,8)	0.151	17940.960	0.067	8916.661	43.35
I_8 (5,6,7)	0.100	1197.025	0.134	594.921	28.90
I_9 (4,5,7)	0.035	42.007	0.014	20.877	10.11
I_{10} (3,4,5)	$0.588x10^{-2}$	6.898	0.027	3.428	1.67
I_{11} (3,5,6)	$0.555x10^{-2}$	6.581	0.058	3.270	1.58
I_{12} (2,4,5)	$0.709x10^{-2}$	8.403	0.057	4.176	2.02
I_{13} (1,2,3,4)	0.015	18.945	0.184	9.416	4.33
I_{14} (1,2,4,5)	0.027	32.375	0.101	16.098	7.80
I_{15} (2,3,5,6)	$0.977x10^{-2}$	11.792	0.023	5.861	2.80
I_{16} (2,3,7,8)	0.060	72.511	0.013	36.038	17.34
I_{17} (3,5,7,8)	0.078	95.280	0.038	47.354	22.54
I_{18} (2,3,4,5,7)	0.238	291.434	0.187	144.842	68.78
I_{19} (1,2,5,6,7)	0.088	10797.410	0.280	536.631	25.43
I_{20} (1,2,3,7,8)	0.264	794.820	0.067	395.026	77.64
I_{21} (1,2,3,4,5,7)	0.288	1077.133	0.125	535.335	82.35
I_{22} (3,4,5,6,7,8)	0.280	345.608	0.051	170.771	82.30
I_{23} (1,2,3,4,5,6,7,8)	0.346	8426.521	0.119	4187.992	100.00

CONCLUSION

It was concluded that selection index I_7 or I_{23} could be used to select buffaloes which will give highest expected aggregate economic gain and will also be efficient.

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STUDIES ON BIOMETRY OF SPERM OF MURRAH BUFFALO BULLS (*BUBALUS BUBALIS*)

Biswajit Roy, P.K. Nagpaul, P.K. Pankaj, T.K. Mohanty, V.S. Raina and A. Mishra

ABSTRACT

Objective of the present study was to measure various biometric parameters of the spermatozoa of Murrah buffalo bulls. Spermatozoa with intact acrosomes were selected and assessed using an immersion lens (1,000X) and standard illumination. The software made it possible to take linear measurements of each spermatozoon: head length, head width, head base, tail length, acrosomal cap length and acrosomal cap width. The results of the morphometric study did not demonstrate the existence of sperm subpopulations. Mean head length, mean head width, mean head base, mean acrosomal cap length and mean acrosomal cap width were 7.59±0.01 µm, 4.91±0.01 µm, 2.47±0.01 µm, 3.46±0.01 µm and 4.51±0.01 µm, respectively. The mean tail length was 56.14±0.01 µm.

Keywords: buffalo, biometry, spermatozoa

INTRODUCTION

Associations of abnormal spermatozoa with bull fertility have yielded varying results. Manual methods of analysis are subjective and highly variable within and between technicians, which may account for these differences. Much time and effort are also spent subjectively evaluating sperm morphology to determine semen quality. For conventional morphology evaluation, sperm are fixed and classified by observers as either normal or abnormal (Barth and Oko, 1989). The percent of total abnormalities or the percent of specific abnormalities are then used as criteria for assessing semen quality. It has been observed that individuals can classify the same sperm differently depending on their interpretation of normal and abnormal morphology (Neuwinger *et al*., 1990). In fact, the same observer may even classify the same sperm differently on successive occasions. Thus, this approach contains subjectivity, is not very repeatable or sensitive and by including the morphologies of dead sperm may incorporate information from cells which cannot participate in fertilization. Other approaches using computer aided image analysis have been proposed to address subjectivity, improve repeatability and enhance sensitivity. To address differences in the shapes of the sperm heads, several investigators have used computer aided image analysis to measure the area, perimeter, length, width and obtained several other measures based on these from sperm heads (Auger and Dadoune, 1993; Gravance *et al*., 1996; Park *et al*., 1997; Aziz *et al*., 1998). Keeping on with this approach the following study was design to evaluate the Murrah buffalo sperm biometry through computer image analysis.

MATERIALS AND METHODS

Twelve healthy, sexually mature and clinically normal Murrah buffalo (MU) bulls of almost similar body weight and age group (nearly 3.0 to 6.5 years) were selected for the study. Semen from the 12 bulls was diluted to 200 X 10^6 sperm/mL. To avoid individual technician variation, one person measured all the parameters from the captured image. Dual staining procedure initially developed by Sidhu *et al.* (1992), which was used with some modification to identify the clear acrosome structure of buffalo spermatozoa. One hundred microliters of semen were mixed with 0.2 percent trypan blue (in TALP medium without BSA) and incubated for 10 minutes on a clean glass slide at

Veterinary Officer, Block Animal Health Centre, Kursoeng, Darjeeling, West Bengal-734203 India.

37°C. After the incubation period, smears of the semen were prepared gently on the glass slides and allowed to dry for 15 minutes at room temperature. A 0.72 percent (W/V) Giemsa stock solution was prepared by dissolving 1 g of Giemsa dye in glycerol-methanol mixture (54:84). One gram of Giemsa was diluted five times with distilled water (final concentration of Giemsa working solution was approximately 0.15%). The smears of spermatozoa previously stained with trypan blue were then stained with Giemsa for 1h at room temperature to evaluate the acrosomal status of the spermatozoa. Smears were dried between the folds of filter paper and stored. The dried smears were studied at 1000X under a light microscope using oil immersion without cover-glass. The slides were used for measurement within a week of preparation. A total of 1100 spermatozoa were measured for the experiment.

Image Analysis Measurements:

Images were randomly selected from each slide by using an Nikon Eclipse E600 microscope attached to an Nikon camera, interfaced to a PC computer, and ACT1 software was used for measurement. The images were obtained by using 100X objectives (oil immersion) in standard light transmission mode (transillumination). Only fresh images were used for the measurements. One speciality of this programme is that stored image cannot be used for measurements. The software was standardized against a decimal scale. One hundred normal sperm were obtained for each bull from different days of semen sample to avoid any day-to-day variation.

Sperm morphology was quantified in terms of the following morphological features: head length (L), width (W), base (B), head area (A), perimeter (P), acrosomal cap length and width, tail length, ellipticity (e), shape factor (SF) (Ostermeier *et al*., 2001). The units for measurement variables were micrometers (μm), the ratios were without units. The head area, ellipticity and shape factor are defined in Equations. (1), (2) and (3), respectively.

$$A = 1.05 - 0.081 \times B^2 + 0.64 \times W \times L$$

Van Duijn (1960) (1)

$$e = \frac{L - W}{L - W} \quad (2)$$

$$SF = (1 - e) \times \frac{P^2}{4\pi A} \quad (3)$$

The head shape was calculated as the ratio of head length and head width (Beatty and Napier, 1960). The width base is defined as the distance between the vertices of the base of the sperm head. Sperm head roundness was calculated as convex perimeter (Hunt *et al*., 1992).

Descriptive statistics (Systat 11.0) were performed on the data to determine normality. Statistical analysis was performed as per standard statistical methods (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The measurements of spermatozoa are presented in Table 2. Individual bull variation was not found for the head length, width, base, head area and shape (width:length), acrosomal cap length and width, tail length, perimeter, ellipticity and shape factor.

Sperm morphometry, in combination with other objective traits, can be useful for developing a fertility index. Associations of abnormal spermatozoa with bull fertility have yielded varying results. Abnormal bull sperm morphology has been correlated with reduced fertility (Sekoni and Gustafsson, 1987; Correa *et al*., 1997). In particular, the occurrence of abnormal sperm head morphology is associated with lower fertility in the bull (Saacke and White, 1972; Sekoni and Gustafsson, 1987). However, a number of other studies have shown no correlation between sperm morphology and fertility (Bratton *et al*., 1956; Linford *et al*., 1976), and clear associations between normal bull sperm morphology and fertility continuing to remain elusive (Johnson, 1997). Any correlations, which have been found between sperm morphology and bull fertility have been based on subjectively performed analyses. Barth (1992) has suggested that these varying results may be due to experimental and classification errors. In the bull, metric criteria for normal sperm head measurements have not been readily applied to fertility assessment; however, large variability in assessing primary sperm abnormalities, including sperm heads, has been found between laboratories (Bishop *et al*., 1954). While

Table 2. Various sperm morphometric indices* of Murrah bulls.

Parameters	Pooled
Head length (µm)	7.59±0.01
Head width (µm)	4.91±0.01
Head base (µm)	2.47±0.01
Acrosomal cap length (µm)	3.64±0.01
Acrosome cap width (µm)	4.51±0.01
Tail length (µm)	56.14±0.18
Head shape (width:length)	0.65±0.00
Ellipticity (e)	0.21±0.00
Head area (μm^2)	24.41±0.05
Perimeter (µm)	19.65±0.02
Shape factor	0.99±0.00
[a]Mean±SE	

manual assessment of bull sperm head morphometry has been associated with fertility (Williams and Savage, 1925) and chromatin structure (Sailer *et al*., 1996), the visual measurement methods employed in these limited studies were extremely laborious or supplied a limited amount of information regarding the overall shape of the sperm head. The wide variation in these sperm head measuring methods makes accurate interpretation of the resulting data difficult; hence, various studies yield contrasting results. Manual methods of analysis are subjective and highly variable within and between technicians, which may account for these differences. Computer-aided sperm head morphometry appears to be a precise method of assessing sperm head dimensions. In the present study, the measurements were taken using Nikon ACT1 software. Our results support the previous work conducted by Aggarwal *et al*. (2007) on Murrah buffalo bulls.

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SEROPREVALENCE OF BOVINE HERPESVIRUS 1 (BHV-1) IN INDIAN BREEDING BULLS OF GUJARAT

Jain Lata, A.N. Kanani, T.J. Patel, J.H. Purohit, M.K. Jhala, H.C. Chuahan and B.S. Chandel

ABSTRACT

The present study was undertaken to screen the sera of cattle and buffalo bulls belonging to five semen collection centres of Gujarat state for the presence of IBRV antibodies employing monoclonal antibody based blocking ELISA (M-ELISA). The indirect fluorescence test was performed to correlate the results obtained by M-ELISA. Out of 89 sera tested by M-ELISA, 26 (29.28%) were positive. A variable rate of seroprevalence was recorded from all the centres included in the study. Further studies, mainly isolation of virus from semen, are warranted. Results indicated the prevalence of IBR infection among cattle and buffalo bulls of semen collection centres of Gujarat state. However, it is emphasized that the cattle and buffalo population of organized farms as well as rural areas of Gujarat State should be screened for IBR to learn the exact picture. Thus, future steps can be taken to control this emerging disease.

Keywords: bovine herpesvirus, seroprevalence

INTRODUCTION

Infectious bovine rhinotracheitis (IBR) caused by BHV-1 of family *Herpesviridae* is amongst the important emerging diseases of cattle and buffaloes in India. BHV-1 is responsible for a variety of clinical conditions in cattle and buffaloes, including infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV), infectious pustular balanoposthitis (IPB), and conjunctivitis and generalized disease in neonates and thus causes great economic losses to the livestock industry (Gibbs and Rweyemamu, 1977).

The infection has serious economic implications for India, which is emerging as the world's biggest milk producer and has the world's largest cattle and buffalo population.

IBR was first recognized in the United States during 1950's and it has since then recognized in many countries throughout the world. In India, the disease was first reported in Uttar Pradesh among cross bred calves in 1976 (Mehrotra *et al.*, 1976). Various workers have since reported widespread seroprevalence and have isolated the virus from different parts of the country, including Gujarat (Renukaradhya *et al.*, 1996; Khan, 2004).

Considering the infectious nature of the disease and its economic implications, a systemic study was undertaken to determine the evidence of IBR in breeding bulls, maintained at semen collection centres employing monoclonal antibody based blocking ELISA and the indirect immuno-fluorescence test.

MATERIALS AND METHODS

I. Serum samples: A total of 89 serum samples were collected from cattle (38) and buffalo bulls (51) stationed at five different semen collection centres of Gujarat state. The separated serum was collected in screw-capped plastic vials and heat inactivated at 56^{o}C for 30 minutes, and Merthiolate (1:10,000) was added in all vials as a preservative. The sera were held at -20^{o}C temperature until use.

Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Anand Agriculture University, Anand, Gujarat – 388001, India.

II. Reference reagents:

a)For M-ELISA: An IBR monoclonal antibody based blocking ELISA kit (Catalog no. B1004-AB01) was made available by courtesy of BV European Veterinary Laboratory, The Netherlands.

b)For indirect fluorescence: An indirect fluorescence kit was made available by VMRD, Inc. Pullman, USA.

Protocol of M-ELISA

All 89 sera were subjected to M-ELISA as per the protocol of the IBR monoclonal antibody based blocking ELISA kit. The protocol is as follow:

1. To all the wells of the microtiter strip, 50 µl of ELISA buffer was added, and then 50 µl of each positive control serum and negative control serum (supplied in kit) was added to positive and negative control marked wells, respectively. Then, 50 µl of serum sample was added to an individual marked sample well of the strip and then incubated for 3 h at 37°C.

2. Microtiter strips were washed with washing solution for atleast 4 times.

3. Then, 100 µl of anti-BHV-1 conjugate was dispensed to all the wells and incubated for 30 minutes at room temperature. Then, the washing step was repeated.

4. Equal parts of substrate A and substrate B were mixed with gentle shaking immediately before use. Then, 100 µl of substrate solution was dispensed to each well and incubated for 15 minutes at room temperature.

5. Then, 50 µl of stop solution was added to each well and mixed by tapping. The absorbence values (OD) were read immediately (within 10 minutes) at 450 nm.

Interpretating the result of M-ELISA

Calculation: Ratio = Sample OD/Negative control OD

The samples were considered to be positive for IBR antibodies when the ratio was equal to or less than 0.6. The sample with ratio equal to or more than 0.7 were considered negative. The samples having ratios between 0.6-0.7 were considered doubtful and retested.

Protocol of Indirect FAT: Out of 89 sera, only 8 sera (6 M-ELISA positive and 2 M-ELISA negative) were tested for presence of BHV-1 antibodies by indirect FAT as per the protocol of kit supplied by VMRD. The protocol is as follow:

1. To an individual marked well of FA Substrate Slide (Catalog no.: 210-88-10-IBR, VMRD) (*i.e.* well for positive control, negative control and field serum) 50 µl serum was added and then the slide was incubated in a humid chamber at 37°C for 30 minutes.

2. The slide was gently rinsed in FA Rinse Buffer (Na_2CO_3 : 11.4 gm; $NaHCO_3$: 33.6 gm; NaCl :8.5 gm; DI/dH_2O up to 1 liter; pH 9.0) and then soaked for 10 minutes in FA Rinse Buffer.

3. To each well, 50 µl labelled anti-IgG FITC conjugate (Catalog no.: 020-1, VMRD) was placed and incubated in a humid chamber at 37°C for 30 minutes.

4. The slide was rinsed in FA Rinse Buffer and then soaked for 10 minutes in FA Rinse Buffer.

5. Slide was mounted with FA Mounting Fluid [glycerol/FA rinse buffer, pH 9.0, (1:1)] and viewed with a fluorescent microscope at 100X-250X and confirmation was done at 400X.

Interpretating the result of Indirect FAT:

In positive control wells, 2-3 positive reactions on positive cells and no reactions on negative cells were seen. While in negative control wells, no reaction was seen on positive as well as on negative cells. In wells containing field serum samples, 2-4 positive reactions on positive cells and no reactions on negative cells were seen.

RESULTS AND DISCUSSION

A total of 89 serum samples from breeding bulls were screened by IBR monoclonal antibody based blocking ELISA and the overall rate of seroprevalance recorded was 29.21 percent . Table 1 represents location-wise, species-wise and breed-wise seroprevalence of IBR in cattle and buffalo bull population.

Similarly, Dhand *et al.*, 2002 reported seroprevalence of 28.76 percent in cattle and

buffaloes in Punjab state. However, contrary to the present findings, Khan (2004) reported the slightly lower rate of seroprevalence of 21.30 percent in the cattle and buffalo population of Gujarat state. While a higher rate of seroprevalence 49.97 percent was reported by Pandita and Srivastava (1993). The variation in this may be due to the sample size, location of the samples collected, inclusion of samples from the sexes, seasons, etc.

Species-wise prevalence was found to be 34.21 and 25.49 percent in cattle and buffalo bulls, respectively. Seroprevalence recorded in cattle was in accordance with the results obtained by Dhand *et al.* (2002). However, contrary to the present findings, Suri Babu *et al.* (1984) reported a higher rate of seroprevalence, 65.78 percent, in cattle from Andhra Pradesh. While Rajesh *et al.* (2003) reported the very low rate of seroprevalence of 14.88 percent in cattle from Kerala state.

During the present investigation, the rate of seroprevalence recorded in buffalo bulls was 25.49 percent, which corroborates the finding of Aruna and Suri Babu (1992) who reported 21.05 percent seroprevalence of IBRV antibodies in buffaloes of Andhra Pradesh. However, Mannickam and Mohan (1987) failed to detect IBRV antibodies in buffaloes in Tamil Nadu.

Of the total 89 serum samples, eight (six M-ELISA positive and two M-ELISA negative) were tested for the presence of IBR antibodies by indirect FAT. One positive and one negative control sera were also placed for the validation of the test result. All the six M-ELISA positive sera were found to be positive by this method and both the two M-ELISA negative sera were negative. Figures 1 and 2 show the immunofluorescent reaction of positive and negative serum, samples, respectively. Thus, 100% correlation was observed between these two methods. However, it is emphasized that a greater number of samples should be tested to draw a meaningful conclusion.

Onisk *et al.* (1989) developed a technique of immunofluorescent assay for the detection of IBR/IPV antibodies and claimed that the technique can be performed to screen a large number of sera samples. Bratanich *et al.* (1990) compared SN, indirect FAT and ELISA for their sensitivity and specificity to detect antibodies to BHV-1 and found high correlation coefficients among all three techniques with a higher sensitivity for the ELISA.

Though the present study is based on limited number of samples only from breeding bulls maintained at various semen collection stations of Gujarat state, it is difficult to reach a meaningful conclusion with respect to breeds, species, locations and regions. However, it is indicative of prevalence of IBRV antibodies in Gujarat state. The prevalence of BHV-1 infection in cattle and buffalo bulls and its serious impact on the livestock industry make it one of the most important infectious agents for livestock. The serological test can be inadequate in the low antibody titer or seronegative latent infection; thus, it is essential that in addition to serological diagnosis, identification of etiological agent should be established or the existence of the antigen before isolation should be revealed by various tests (Duman *et al.*, 2007). Moreover, further systematic seroepidemiological and isolation studies are warranted to find out the actual status of IBR in Gujarat state and elsewhere in India. Thus, further steps can be taken to control this emerging disease.

Table1. Seroprevalence of IBRV/BHV-1 in breeding bulls by M-ELISA.

Attributes	Numbers tested	Number positive	Percent positive
[A] Location:			
Himmatnagar	17	01	5.88
Surat	14	03	21.43
Rajkot	30	14	46.67
Mahesana	24	05	20.83
Anand	04	03	75.00
Total	89	26	29.21
[B] Species-wise:			
Cattle	38	13	34.21
Buffalo	51	13	25.49
Total	89	26	29.21
[C] Breed-wise (Cattle) :			
Cross bred	21	1	4.76
Gir	15	11	73.33
Kankrej	2	1	50
Total	38	13	34.21
[D] Breed-wise (Buffalo):			
Mahesani	34	7	20.59
Jafrabadi	10	3	30
Surti	7	3	42.86
Total	51	13	25.49

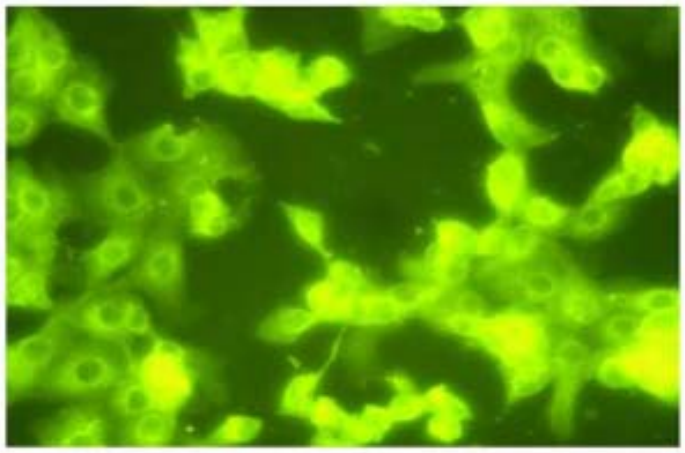

Figure 1. Immunofluorescent staining reaction with field serum sample antibody-antigen complex of BHV-1 infected MDBK cells monolayer on well and FITC antibovine IgG conjugate. Note the bright fluorescing specific reaction on infected cells as compared to normal cells (400X)

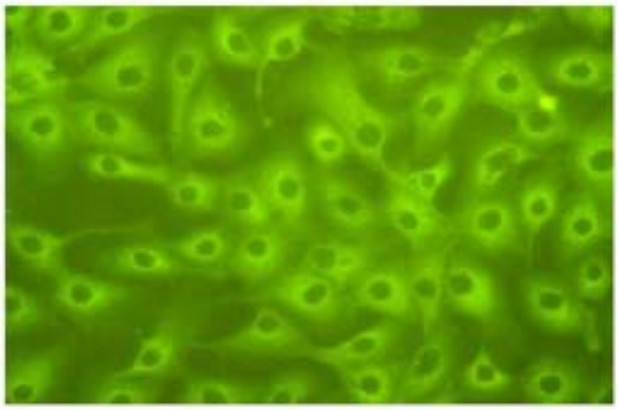

Figure 2. Immunofluorescent staining reaction with negative control antibody-antigen complex of BHV-1 infected MDBK cells monolayer on well and FITC antibovine IgG conjugate. Note the absence of fluorescence (400X).

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HEPATOCELLULAR CARCINOMA IN AN INDIAN BUFFALO

Selvam, G., M. Swamy and Y. Verma

ABSTRACT

A case of hepatocellular carcinoma in a she buffalo is reported. Since hepatocellular carcinoma is very rare in Indian buffaloes, the present communication may serve as a valuable record regarding the incidence and pathological alterations noticed in the affected liver.

Keywords: buffalo, hepatocellular carcinoma, pathological alterations

INTRODUCTION

Hepatocellular carcinoma (HCC) is a type of primary liver cancer that frequently originates as a sequel to chronic liver diseases. Among animals, it is more common in cattle and sheep, whereas in dogs and cats it occurs with lesser frequency than cholangiocellular carcinoma (Jones *et al*., 1997). However, perusal of available literature suggests very low incidence of HCC in Indian buffaloes (Gupta *et al*., 2003). The present communication describes the pathological lesions of HCC observed in postmortem examination of a 4-year-old she buffalo.

CASE HISTORY, GROSS AND MICROSCOPIC PATHOLOGY

The animal had the clinical history of prolonged anorexia, reduced weight gain, pyrexia and sudden death after a continuous treatment for over 3 weeks. Grossly, the carcass looked highly emaciated with sunken eyeballs, and the liver showed hepatomegaly with two large grayish white firm and hard mass at the ventral surface. On cut surface, the parenchyma revealed numerous small white nodules all over the liver. The representative tissue pieces containing liver tissue along with the tumorous growth were collected in 10 percent neutral buffered formal saline and processed for routine hematoxylene and eosin staining (Gridley, 1960). The microscopic examination showed the separation of liver tissue and the tumorous mass by a thick fibrous connective tissue layer which was infiltrated with numerous mononuclear inflammatory cells (Figure 1). Normal structural components like, portal triad, central vein and hepatic lobulation were absent in the tumorous mass. The neoplastic hepatocytes were well-differentiated and arranged as acinar or alveolar masses of approximately 7-9 cells thick and were separated by fibrous septa (Figure 2 and 3). Focal areas of necrosis, cystic degenerative changes (spongiosis hepatis) with infiltrated inflammatory cells (Figure 4), distension of lymph vessels were observed. The nuclei were pleomorphic with clear nuclear membrane and approximately 2-3 prominent nucleoli were noticed in almost all the nucleus. The cytoplasm was more basophilic than the non-neoplastic hepatocytes and showed a decreased cytoplasmic to nucleus ratio. However the mitotic figures were less in number.

DIAGNOSIS AND DISCUSSION

Hepatocellular carcinomas in animals are uncommon (Jubb and Kennedy, 1970). Grossly, hepatocellular carcinomas typically consist of gray white or yellowish brown tissue that is subdivided into lobules by multiple fibrous bands (Maclachlan and Cullen, 1995). whereas microscopically, the malignant hepatocytes shows an abnormal growth pattern and characteristically arranged as a trabecular or acinar pattern or mixtures of both (Maclachlan and Cullen, 1995; Jones *et al*., 1997

Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh-482001, India.

and Gupta *et al*., 2003). Spongiosis hepatis is a multilocular cystic lesion containing finely granular or flocculent eosinophilic material arise from sinusoidal stellate cells and are more often found within hepatocellular proliferative lesions like adenomas or carcinomas (Narama *et al*., 2003).

Since the incidence and the pathological alterations observed were in accordance with previous observations, the present case was diagnosed and recorded as a rare case of hepatocellular carcinoma in an Indian buffalo.

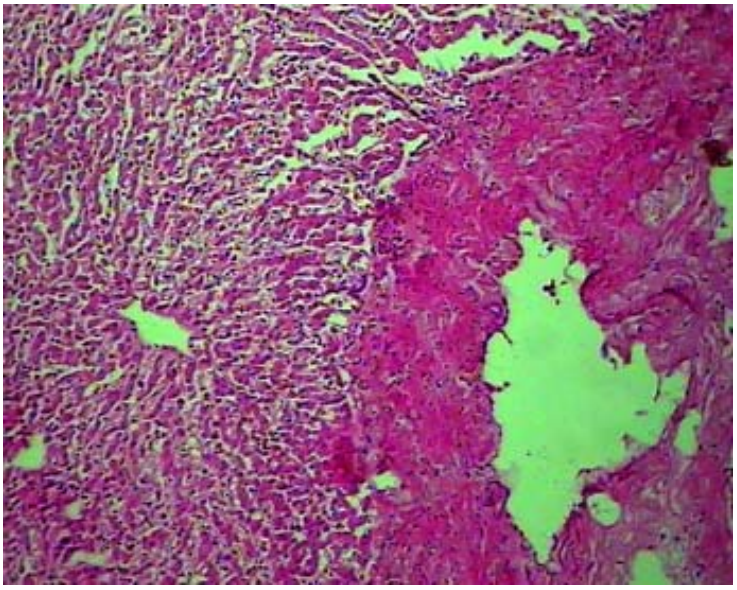

Figure 1. Microphotograph showing non-neoplastic hepatocytes with radiating arrangement in left and thick fibrous septa infiltrated with inflammatory cells on right H & E 100x.

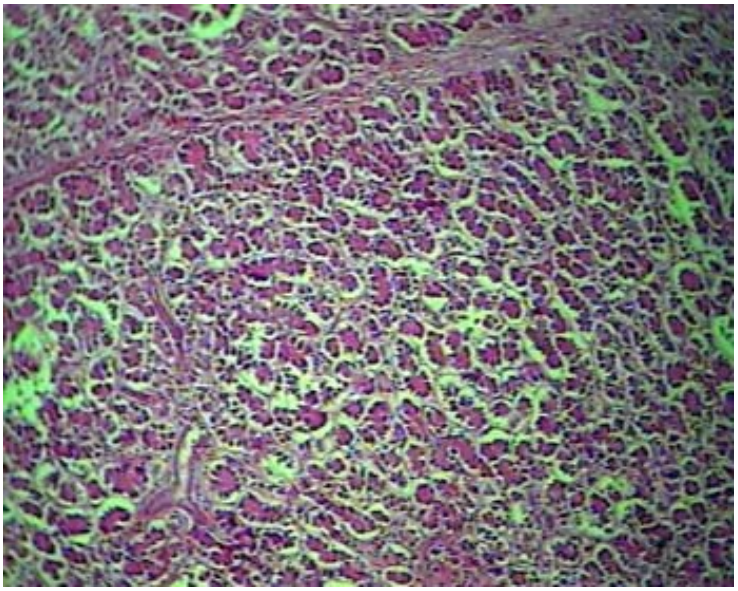

Figure 2. Microphotograph of tumor mass showing abnormal acinar pattern of the neoplastic hepatocytes H & E 100x.

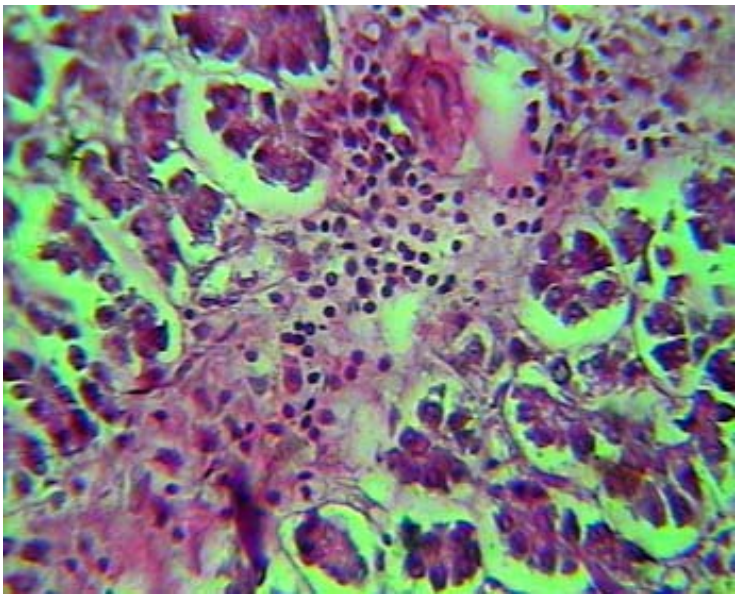

Figure 3. Microphotograph showing acinar pattern of hepatocytes and infiltration of mononuclear inflammatory cells H & E 400x.

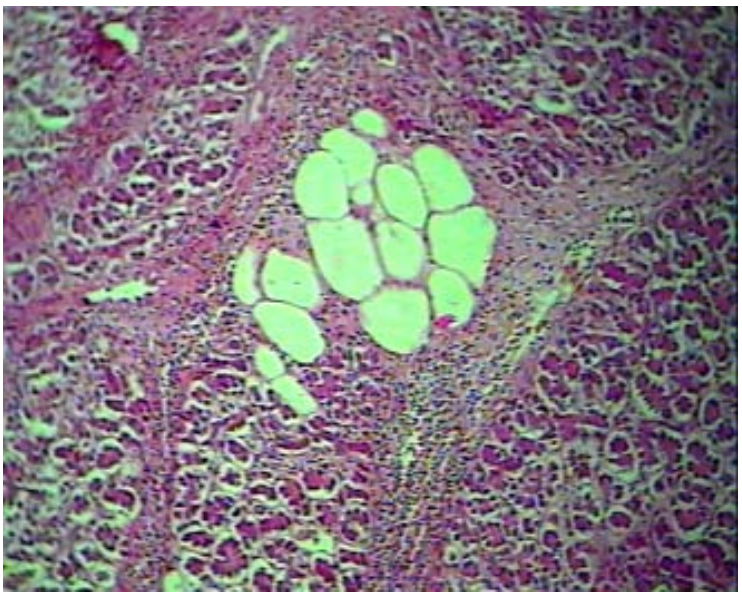

Figure 4. Microphotograph showing cystic changes (Spongiosis hepatis) infiltrated with mononuclear inflammatory cells H & E 100x.

*Continued on page 179

MACRO MINERAL PROFILE IN FORAGE, BLOOD PLASMA AND URINE OF GRAZING BUFFALOES WITH RESPECT TO SEASONAL VARIATION

Zafar Iqbal Khan, Muhammad Danish, Kafeel Ahmad

ABSTRACT

This investigation was carried out to assess the levels of some minerals in blood plasma, and urine of buffaloes and forage consumed by the these grazing ruminants in the central region of Punjab Pakistan using apparently health animals during two consecutive seasons of the year 2006. Blood plasma and urine samples were obtained from the animals thrice during each season, and analyzed for calcium, magnesium, potassium, and sodium. The results showed that concentrations of all the minerals studied in plasma were comparable in both the seasons with the exception of sodium, which was lower in winter, and potassium, which was higher in the same season. For urine samples, concentrations of all the minerals were higher in summer than that in winter. Analysis of forages collected showed that the concentrations of all the minerals were higher in summer than that in winter, showing the need of supplementation during this season for grazing animals at the place where the livestock were being reared.

Keywords: seep, grazing, minerals, status, plasma, forage, pasture

INTRODUCTION

Herbivores animals under natural grazing conditions obtain their minerals from forage plants. Inadequate mineral intake leads to reduced productivity. The adequacy of the diet in essential minerals can be determined by chemical analysis of animal body tissue and fluids, and of forages which are the sole sources of minerals for the requirements of the animals.

In Pakistan, ruminant production depend largely on the use of natural pastures throughout the year. Only rarely, however, can pasture forages completely satisfy all minerals requirements (McDowell *et al*., 1993; Khan *et al*., 2006, 2007). There is need for information on this aspect in Pakistan, in which problems of mineral nutrition exist, before recommendations for specially formulated mineral supplements can be made. The use of mineral supplements without regard to local conditions can cause mineral imbalance and is therefore, likely to impede rather than promote improvement in livestock production. The livestock ranch in the province of Punjab, on which the present investigation was carried out, is the driest region for animal ranching and only fragmentary data is available concerning the mineral status of livestock and forages available at that ranch.

The main objectives of this investigation were, therefore, to determine mineral imbalances and particularly to find out the effect of season on the levels of some essential minerals in forages and animal fluids, in order to assess the need of formulation of mineral mixes for livestock being reared at that ranch.

MATERIALS AND METHODS

Forage sampling

Five representative forage samples were collected thrice, in the summer and winter seasons of the year 2006.The forage samples consisted of both graze and browse species, and were collected after careful observation of grazing pattern of animals Animals were watched grazing and samples representing the diet of the animals were clipped using stainless steel scissors and plastic gloves. The grazing area was stratified according to variations in plants used as forages in order to obtain a representative sampling pattern and forage plants

Department of Biological Sciences, University of Sargodha-Pakistan.

were taken from these sampling positions in the grazing pasture at a distance of 60 m. Forage samples were dried in an oven at 60 ^{o}C for 48 h, ground using a hammer mill and passed through a 1-mm sieve (Fick *et al*.,1979). The ground-up samples were stored in closed plastic bags awaiting chemical analysis.

Animal sampling

Blood plasma and urine samples were taken from forty health buffaloes receiving no mineral supplements at the time of investigation and grazing on the veld pastures only. Blood samples were collected from the jugular vein into 10 ml vacutaioner tube having sodium heparin as an anticoagulant. The blood was centrifuged within 2 h of collection, at 3000 rpm for 25 minutes, to obtain plasma, and the harvested plasma was stored frozen until chemical analysis for minerals was undertaken. Urine samples were obtained using the catheter in the female genital tract after washing it with soap carefully; the urine contents were poured in plastic bottles with screw caps. The urine was preserved by adding formaldehyde. All the animal samples were taken thrice concurrently with forage sampling during each season.

Chemical and statistical analyses

The mineral concentrations of all the samples were estimated following the procedures of Fick *et al*.(1979) and Mpofu *et al*.(1995).

The data were analyzed using a split-plot design (Steel and Torrie 1980). Differences among means were ranked using Duncan's New Multiple Range Test (1955).

RESULTS

The mineral contents of forages, blood plasma, and urine samples are summarized in Table 1.The forage, plasma, and urine contained higher levels ($P<0.001$) of Ca, Mg, K, and Na during summer than during winter. The variation in Ca and Na in the forage were found to be significant ($P<0.001$), with higher levels of these macro elements during summer compared to during winter. However, forage had statistically non-significant ($P>0.05$) levels of Mg and K during both seasons; contents of these electrolytes were higher during summer than those during winter though the difference was not statistically significant. The depression in Mg concentrations in the plasma between the seasons was not statistically significant ($P>0.05$), with non-statistically significant higher levels of these minerals during summer than during winter, while the reverse was true for Ca, K and Na. However, the buffaloes urine had significant fluctuations in concentration of all macro-minerals between both seasons with higher levels in summer and lower in winter.

DISCUSSION

The functions of the minerals in animal physiology are interrelated: seldom can they be considered as single minerals with independent and self-sufficient roles (Bonhomme, 1990; Usaida *et al*.,1991; Ozdemir *et al*., 2006). The mineral elements are not synthesized in the body but are supplied by the feed. Their concentrations in the body fluids will therefore depend on the mineral contents of feed and forage, the level of dietary sources intake, and the availability of minerals (Gomide,1978; Kamalu *et al*., 2006; Khan *et al*.,2007). The concentrations in the body fluids will then depend on absorption from the gut, metabolic usage, hormones and excretion (Littledike and Goff, 1987). Plant forages make up the bulk of the diet consumed by the grazing livestock on both natural and improved pastures. Many environmental and plant factors affect the mineral concentrations of forage plants; these include, species or strain/ variety, soil type, the climatic of seasonal conditions during plant growth, stage of maturity of forage plants and other management practices.

The data reported here indicate most of the macro-minerals studied are higher in the summer season both in the animal fluids and forages compared to those during winter season. Some differences were also found in the mineral content of forage and plasma but there were much greater differences observed in the mineral concentrations

in the urine samples between the seasons, which would be expected to parallel to the forage mineral concentrations and physiology of the alimentary canal of the livestock. It has been reported that the distribution of the different essential minerals and ions between the alimentary fluid and particles of diet is greatly influenced by acidity and that the stage of plant maturity adversely affects most minerals in forage (Underwood, 1981; Littedike and Goff, 1987; Khan *et al*., 2005).

The lower concentration in the buffaloes; urine during the winter might have been as a result of decreased levels in forage plants due to the advanced level of maturity during this season, as when this study was carried out, the forge plants had become brown, dry, and withered, so having the lower levels of mineral content.

Urinary concentration of micro-minerals usually reflects the quantity excreted after the need of the body have been met. When intake is inadequate, there is almost complete re-absorption in kidney and very little in the urine. In this investigation, the fact that the concentration of micro-minerals were higher in summer than in winter season was probably due to a higher amount available for absorption during this season from forages consumed by the animals studied, and some other factors may possibly be involved as a result of hormonal control of the animals' body metabolism (Silbert and Mc Farlane, 1971; Dziuk, 1984).

Table 1.Concentrations of macro minerals (Mean±SE) in forages, blood plasma, and urine sample at buffaloes ranch

Elements	Season	Forage (%)	Plasma (mmol/L)	Urine (mmol/L)
Ca	Summer	0.18± 0.15[a]	4.12 ± 0.22[a]	4.95 ± 1.26[a]
	Winter	0.14 ± 0.11[b]	3.88 ± 0.20[b]	2.76 ± 1.18[b]
Mg	Summer	0.23 ± 0.05[a]	0.84 ± 0.08[a]	3.90 ± 0.44[a]
	Winter	0.20 ± 0.07[a]	0.76 ± 0.05[a]	2.45 ± 0.38[b]
K	Summer	2.22 ± 0.12[a]	5.87 ± 0.14[a]	132.42 ± 12.24[a]
	Winter	1.17 ± 0.15[b]	3.15 ± 0.20[a]	88.90 ± 8.45[b]
Na	Summer	0.048 ± 0.004[a]	165.75 ± 8.92[a]	78.66 ± 11.75[a]
	Winter	0.026 ± 0.003[b]	144.52 ± 12.25[b]	45.35 ± 15.16[b]

Critical value for forage: Ca = 0.20%, Mg = 0.12%, K = 0.5%, Na = 0.09% (NRC 1985)[19]
Critical value for plasma: 2 mmol/L, Mg = 0.83 mmol/L, K = 5.13 mmol/L, Na = 130.43 mmol/L (Mc Dowell *et al*. 1984, Miles *et al*. 2001).
a-b = means with the same letter do not differ significantly at (P≤ 0.05).

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HAEMATOLOGICAL ALTERATIONS DURING DIFFERENT SPONTANEOUS LIVER LESIONS IN BUFFALOES

G.P. Jatav, U.K.Garg and Supriya Shukla

ABSTRACT

The present investigation was carried out to study of spontaneous occurrence of various pathological conditions in the livers of buffaloes, with possible correlation to haematological parameters. Examination of livers and blood from a total number of 510 buffaloes ranging from 4 to12 years of age were examined for liver affections and haematological alterations. The material was collected from Cantonment Board Slaughter House, Mhow (M.P). The mean values of packed cell volume (PCV), haemoglobin (Hb), and mean corpuscular volume and total plasma protein in all pathological conditions of the livers were higher than normal whereas in differential leucocytes count (DLC), lymphocytosis was revealed during the different pathological conditions of liver, except in case of suppurative hepatitis, hyaline degeneration and cloudy swelling in which the lymphocytes were within the normal range.

Keywords: haematology, pathological conditions, liver, buffalo, differential leucocyte count, PCV, Hb, MCV, total plasma protein.

INTRODUCTION

The buffalo is the predominant domestic animal for milk and meat production. On average, buffaloes are about four times as productive as average indigenous cows in India. India has the world's best dairy buffalo breeds and provides superior buffalo germplasm to several countries of the world (Kaikini, 1992). Buffaloes are very prone to various bacterial, viral, fungal, parasitic diseases and other diseases of diverse etiology. The liver is the first organ of the body that undergoes pathological changes when an animal suffers from acute infection and the last organ to assume normalcy (Gracey, 1981). The present investigation was carried out on the livers of buffaloes to investigate the possible relationships of certain haematological values, viz. differential leucocyte count (DLC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV) and total plasma protein.

MATERIALS AND METHODS

The materials for the present study comprised liver and blood samples obtained from buffaloes slaughtered at the Cantonment Board Slaughter House, Mhow, (M.P.) which had been brought from the different parts of the Malwa region as the source of meat. A total of 510 buffaloes ranging from 4 to 12 years of age were examined for liver lesions. To determine the haematological changes, the blood samples were collected during slaughter of buffalo in a sterile vials containing anticoagulant, ethylene diamine tetra acidic acid (EDTA) @ 2 mg/ml of blood. The haematological parameters DLC, PCV, Hb, MCV and total plasma protein were carried out as per the procedures mentioned by Jain (1986).

RESULTS AND DISCUSSION

The haematological changes observed during the present study, are as shown in Table 1. Lymphocytosis was observed during the different pathological conditions of the liver (56$\pm$7.07

Department of Veterinary Pathology College of Veterinary Science & A.H., Mhow, M.P., (INDIA).

to71±4.94) except in case of suppurative hepatitis, hyaline degeneration and cloudy swelling, in which the lymphocyte count was almost same as normal values described by Jain (1986). On the contrary, the PCV was found to be increased in all the conditions, ranging from 34.25 to 50.67 as against the normal value of 31.0 (Jain, 1986). The values of Hb under pathological conditions encountered in the present study were compared with normal ranges, and it was evident that the values of Hb were higher in all pathological conditions of the liver (i.e. 14.6-16.2 g/dl), except in case of atrophy, cloudy swelling, and cirrhosis, in which the values were within the normal range (9-13.5 g/dl) observed by Jain (1986).

In the present study, the plasma protein values were much higher (9.93-13.57 g/dl) than the mean values (6.8-7.7 g/dl) mentioned by Brar *et al.* (2000), which reflected a significant increase during the different pathological conditions of the liver of buffaloes as compared to normal. In the present findings the MCV values were significantly higher (59.62–83.7 g/dl) than the normal mean values. These values were much higher than the range (42-58 g/dl) given by the Brar *et al.*, (2000). The values of MCH and MCHC were also higher than the normal range except in case of hyaline degeneration, in which the MCHC was within the normal range.

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Table 1. The mean values of haematological alterations in buffaloes during different pathological conditions in liver (Mean ± SE).

Pathological conditions/ Blood Parameters	No. of ca.	PCV (%)	TPP (g/dl)	Hb (g/dl)	MCH (pg)	MCV (f l)	TLC ($\times 10^3$/cu. mm)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	Basophils (%)
Atrophy	3	35.67±1.20	13.26± 0.67	13±0.87	26.6±3.96	71.2±16.40	6.02±0..67	29±11.02	60.67±11.71	2..66±1.44	6..33±0.88	1.66±0.66
Hypertrophy	4	40.5±1.55	13.37±0.32	14.6±1.03	22.78±2.20	64.2±8.09	7.85±0.90	18±3.67	71±4.94	3.5±1.70	6±0.91	1.5±0.28
Cloudy Swelling	4	34.25±6.0	12.9± 0.34	12.28± 1.84	24.31± 2.98	65.87± 3.95	8.65±0.66	31.25±8.38	52.75±5.97	5.25±1.70	9.75±2.7	1.±0.70
Fatty changes	6	41.67±1.95	12.21± 0.91	16.2± 0.94	24.92 ± 2.56	63.6±5.08	6.6± 1.41	29.67±3.30	58.33±2.25	5± 1.69	6.16± 1.45	0.83±0.30
Amyloidosis	5	43.2±5.13	12.28± 1.20	13.5± 0.87	25.74±1.47	80.1± 264	6.67± 0.69	40.2±5.8	51.4±4.20	3±1.30	4.6± 1.16	1.2±0.20
Hyaline degeneration	3	50.67±3.53	11.93±2.16	14.7±0.76	24.49±2.30	83.7± 1.95	5.56±0.24	41±6.81	50±4.04	4±2.08	4.33±1.20	1.33±0.33
Cirrhosis	2	35.5±2.5	13.15±0.35	13±0.00	21.67±0.27	59.62±10. 39	8.85±2.00	39±5.01	56±7.07	1.5±1.50	3.5±1.50	1±0.00
Suppurative Hepatitis	2	41±4.0	12.65±0.26	15.6±1.20	23.01±1.23	60.5±4.49	7.77±0.00	41.5±0.5.	53±1.00	2±1	1.5±0.5	1.5±0.5
Toxic Hepatitis	12	42.25±1.88	12.37±0.50	15.5±0.81	22.42±1.39	60.7±3.17	8.9±0.834	25.17±3.15	63±2.42	4±1.10	6.83±1.47	1±0.27
Fascioliasis	5	36.4±2.01	9.93±1.66	14.7±1.20	27.12±0.68	59.92±9.05	9.93±1.68	23.2±2.45	64±2.21	4.2±1.56	2.7±1.01	1.4±0.24
Hydotidosis	6	39.67±2.56	13.25±0.39	15.42±1.25	26.62±1.72	66.49±3.66	6.95±0.63	29.66±6.74	61.5±5.61	3.5±1.43	5.83±1.37	1±0.23
Pigmentation	4	39.75±3.94	13.57±0.42	15.58±1.57	25.2±1.99	64.79±6.17	6.92±1.51	26.75±4.12	66.75±4.64	2±0.35	5.5±0.86	1±0.00
Neoplasm	3	40±1.73	12.13±0.133	14.96±1.32	22.67±1.21	61.1±4.48	9.78±2.21	31.67±6.06	57.33±2.02	3.67±2.18	6.33±2.18	1±1.00

** Jain (1986) and Brar *et al.* (2000).

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